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14. ABSTRACT: This is a study of the effects of ibuprofen, a non-steroidal anti-inflammatory drug (NSAID), on the osteogenic response to 9 months of exercise training in healthy, premenopausal women, aged 21 to 40 years (N=102). The hypotheses are: H1a: taking short-acting NSAIDS before exercise will diminish increases in bone mineral density (BMD) in response to exercise training H1b: taking short-acting NSAIDS after exercise will not diminish the increases in BMD in response to exercise training Participants take either ibuprofen (400mg) or placebo capsules before and after each exercise session. Women are randomized to three treatment arms: 1) NSAID before exercise, placebo after exercise (NSAID/placebo; n=34); 2) placebo before exercise, NSAID after exercise (placebo/NSAID; n=34); and 3) placebo before exercise, placebo after exercise (placebo/placebo; n=34). One hundred thirteen women completed baseline testing and were randomized to treatment. Final follow-up testing was completed approximately 7 months ago and most sample analysis has been completed. Re-analysis of some samples and review of the database continues for quality assurance. Manuscript preparation is underway. These studies could lead to the development of new strategies to reduce the incidence of, and treatment for, stress fractures that occur in response to vigorous physical activity.

15. SUBJECT TERMS
Exercise, stress fracture, ibuprofen, prostaglandins, bone mineral density, estrogen

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INTRODUCTION:

The primary aim of this randomized, double-blinded, placebo-controlled trial was to determine the effects of NSAID (ibuprofen) use on the osteogenic response to 9 months of exercise training in 102 women. The scientific rationale for this study centers on the knowledge that the osteogenic response to mechanical stress is a prostaglandin (PG)-dependent process and that NSAIDs inhibit PG synthesis. There is evidence that regular NSAID use inhibits the normal bone formation response to mechanical loading, increases risk of fracture, and impairs bone healing. The approved statement of work for this project included 4 years for recruiting, testing, and training subjects and completing sample assays, data analysis, and manuscripts. Completion of the trial took longer than expected, but a no-cost extension was requested to facilitate publication of results.

BODY:

The study was designed to address the following hypotheses (H):

H1: Consumption of a short-acting NSAID **before** exercise will diminish the increases in BMD of the total body, proximal femur, and lumber spine in response to 9 months of exercise training.

H2: Consumption of a short-acting NSAID **after** exercise will not diminish the increases in BMD of the total body, proximal femur, and lumber spine in response to 9 months of exercise training.

The original Statement of Work included the following objectives:

Major objectives:

- Year 1
  - Start enrollment in month 3 of year 1
  - Perform all tests and procedures at baseline and 3, 6, and 9 months of intervention in each participant, as outlined in the protocol
- Year 2
  - Continue enrollment and performance of tests and procedures
  - Start assays of biochemical markers of bone turnover and sex hormones in month 8, after roughly 20 subjects complete the study
- Year 3
  - Complete enrollment by month 12
  - Continue performance of tests and procedures
  - Continue biochemical assays
- Year 4
  - Complete all tests, procedures, and biochemical assays
  - Perform data analyses and prepare manuscripts

The project took longer than expected to complete, requiring a 1.5-year no-cost extension. Factors that contributed to the delays included the moving of the PI's laboratory (and exercise training facility) to a new off-campus location, and some facility issues at the new location (i.e., poor temperature regulation during the summer months, which slowed our recruitment and, we believe, resulted in an increased rate of attrition). Nevertheless, we were successful in enrolling more volunteers than originally planned (113 vs 102) and successfully completed all of the

objectives, with the exception that the results of the study have not yet been published. The data will be published when final statistical analyses have been completed (addressed below).

Recruitment, enrollment

Figure 1 illustrates the flow of volunteers through the study. The attrition rate of 34% was higher than the projected rate of 25%. More women were randomized to treatment (n=113) than originally proposed (n=102) to help offset the attrition. It was proposed that primary analyses would be based on compliance, rather than intent-to-treat, analyses because the study was a physiologic, rather than a therapeutic, trial. It was estimated that 19 finishers per group would be required to achieve $\beta<0.020$ (i.e., power of 80%). The racial and ethnic characteristics of the study participants are similar to those that were projected, which reflect the demographics of the Denver metropolitan area (Table 1).

All statistical analyses presented in the report should be considered preliminary. The dataset remains in the hands of the GCRC biostatistician, who will conduct all final analyses. It was expected that final analyses would have been completed by the time of this report. It has taken longer than expected for two reasons. First, because of the biostatistician's commitment to assist in the development of an institution-wide center grant application (i.e., NIH Clinical and Translational Science Award) and another center grant, analysis of the dataset for this project was reduced to a lower level of priority. Second, the preliminary analyses yielded a finding that was not expected, as discussed below. To ensure that we are interpreting these findings appropriately, further analyses are necessary.

Randomization, baseline characteristics

The randomization process was successful in achieving equitable distribution across the treatment groups, with stratification by use of contraceptive therapy. Table 2 includes the baseline characteristics of the full cohort, by group. There were no significant differences among the groups in any of the characteristics. It should be noted that dietary data were available for only 18 participants in each of the treatment groups. Diet record collection and analysis was performed through the General Clinical Research Center (GCRC) bionutrition core. During the final data quality assurance check, it was determined that the records for the remainder of the participants either had not been obtained or had not been obtained correctly. The available data suggest that energy intake and composition was likely similar among the groups.

The baseline characteristics of the 73 participants who completed the intervention and provided follow-up data are presented in Table 3. The characteristics of the groups changed minimally as a result of attrition (i.e., Table 2 vs Table 3) and there remained no significant differences among the groups in any of the characteristics. However, the rates of attrition may have been different among the groups. Therefore, attention will be directed to controlling for unequal group sizes in data analysis.

Of the 73 women who completed the intervention, preliminary analyses indicate that 54 were compliant to the intervention, defined as meeting at least 80% of the prescribed intervention. There were no significant differences in baseline characteristics among the groups, but differences in group size persisted (Table 4). The most common reason for dropping out of the study was lack of time to attend the exercise sessions, and this was the case across all treatment groups. It does not appear that the higher rate of attrition in the placebo/ibuprofen arm was treatment-related.

Changes in outcomes in response to exercise training

BMD

Changes in outcomes are reported for participants who were compliant to the intervention. Primary outcomes for the trial were changes in BMD in response to exercise training, and these are depicted in Figure 2. At all skeletal sites, the largest increases in BMD occurred in the placebo/ibuprofen group; differences between groups were significant for all hip regions, but not for the lumbar spine ($p = 0.18$). However, although we hypothesized that there would be differences in BMD among the groups, these preliminary results are not consistent with the hypothesized differences. It was predicted that both the placebo/ibuprofen and placebo/placebo groups, rather than just the placebo/ibuprofen group, would have increases in BMD of 1.5-2.0%. Analyses that adjust for potential mediators of the BMD response to exercise, such as use of contraceptive therapy, sex hormones, or changes in body composition, have not yet been performed. We will specifically try to determine whether there is a reasonable explanation for why the placebo/placebo group failed to have more robust increases in BMD.

Body composition

Changes in body composition in response to exercise training are depicted in Figure 3. Neither the changes in fat mass ($p = 0.48$) nor the changes in fat-free mass ($p = 0.42$) were significantly different among the groups.

Bone turnover

Serum markers of bone turnover (CTX – resorption; BAP - formation) were measured at 3 times points during the menstrual cycle (early follicular, once; mid-luteal, twice) in the month before the start of the exercise program and in the final month of the exercise program (Figure 4). Statistical analyses of these data have not been completed, but no remarkable differences among the groups are apparent.

The average of the 3 samples measured at baseline and the average of the 3 samples measured in the final month of the exercise program were used to determine the relative changes over the period of intervention (Figure 5). These data may provide insight into the mechanisms underlying the changes in BMD. Preliminary analyses do not indicate significant differences among the group in change in CTX or BAP in response to exercise. However, the increase in CTX in the placebo/ibuprofen group (i.e., the group that had the largest increases in BMD) was less than half as great as in the other groups. NSAIDs have been found to inhibit bone resorption under certain conditions,[1] but we are unaware of any evidence to explain why this would be different if the NSAID was taken before or after exercise. Although it is generally believed that exercise has a suppressive effect on bone resorption,[2] increases in bone turnover have been reported.[3] Because estrogen has potent anti-resorptive effects,[2] we will examine whether the increases in bone turnover can be explained by hormone changes.

Sex hormones and gonadotropins

Sex hormones (estradiol, testosterone, progesterone, sex hormone binding globulin (SHBG)) and gonadotropins (luteinizing hormone (LH), follicle stimulating hormone (FSH)) were measured at the same time intervals as the markers of tone turnover (Figures 6-8). Relative changes in sex hormones over the intervention period were calculated in a similar manner as changes in bone markers. Preliminary analyses do not indicate that there were significant differences among the groups in the changes in sex hormones. However, an intriguing

preliminary finding is that the group that had the largest increases in BMD (placebo/ibuprofen) was the only group that did not have decreases in progesterone and estradiol. NSAIDs have been found to alter ovarian function and sex hormone levels in mammals,[4] but it is not clear why timing of the use of ibuprofen (relative to the performance of exercise) would be a possible determinant of sex hormone changes in the current study (i.e., why the ibuprofen/placebo and placebo/ibuprofen groups would respond discordantly). Also, because the dose of ibuprofen in the study was very low, it is not clear that there should be an NSAID effect on sex hormones. Analyses will be conducted to determine if changes in sex hormones mediated the changes in BMD.

Table 5 presents a summary of dietary and VO_2max results. These data indicate that energy and nutrient intake remained relatively constant over the period of study (in the subset of participants for whom data were available) and that the endurance component of the exercise program was sufficiently intense to generate small increases in VO_2max.

KEY RESEARCH ACCOMPLISHMENTS:

All aspects of the project have been carried out to completion with the exception of final data analyses and publication of results. The preliminary data suggest that use of ibuprofen may alter the BMD adaptation to exercise, though possibly not in the manner hypothesized. Consistent with our hypotheses and with studies of animals and cells,[5-7] taking ibuprofen after exercise resulted in more favorable changes in BMD than taking ibuprofen before exercise. However, interpretation of these results is complicated by the small changes in BMD that occurred in the placebo/placebo group; it was expected that the most robust BMD changes would occur in this group. Although all participants were asked to minimize use of non-study NSAIDS, it is possible that this group had more NSAID use because they did not get relief of exercise-related discomforts via the study drugs. If there was a need for pain relief, participants were instructed to use acetaminophen rather than NSAIDs, because it is thought the acetaminophen does not inhibit COX activity (as do NSAIDs). However, Trappe and colleagues reported that both ibuprofen and acetaminophen attenuated exercise-induces increased in prostaglandins in skeletal muscle, and both blunted the increase in muscle protein synthesis.[8,9] Further, in a recent study, both ibuprofen and acetaminophen use was associated with increased fracture risk; the authors acknowledged that the mechanism by which acetaminophen would increase fracture risk is not clear.[10] It will not be possible to evaluate the potential confounding effects of acetaminophen use in the current study because acetaminophen use is not known.

REPORTABLE OUTCOMES:

The results of this trial have not yet been published. The unexpected changes in BMD have complicated the interpretation of data. Preliminary analyses suggest that taking ibuprofen after exercise may beneficially influence the adaptation of bone. However, the investigators want to be very cautious in publishing the results of the study, because there is a concern that the findings could promote the use of ibuprofen or other NSAIDs. Further data analyses, controlling for use of contraceptives, volume of exercise, and other factors that may have influenced the bone response, will be carried out to help ascertain whether the BMD changes were truly related to ibuprofen use. Regardless of the results of these analyses, it must be remembered that this was the first study to evaluate the effects of NSAIDs on changes in bone in humans, and additional studies will be necessary to either confirm or refute the findings. It will be very important to publish the findings of the current study to stimulate such research. We will proceed with the publication of the results after the biostatisticians have completed the data analysis.

CONCLUSIONS:

Conclusions cannot yet be drawn because final data analyses have not been completed. This remains a completely novel area of investigation in humans. We are not aware of any intervention studies that have evaluated whether non-steroidal anti-inflammatory drugs (NSAID) impair the osteogenic response to mechanical loading in humans. The importance of the timing of NSAID administration relative to mechanical loading, which had been evaluated in one animal study at the time the original grant application was prepared,[5] has been reinforced by a second study.[7] However, as discussed in a very recent paper on COX inhibition and loading of bone in rats,[11] the mechanisms by which COX activity influences the response of bone to mechanical stress remains uncertain. Although the current study has not yet generated publications, we are cogently aware of the importance of disseminating the results through peer-reviewed publications and will do so when analyses have been completed.

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APPENDICES:

Figure 1. Study participant flow chart

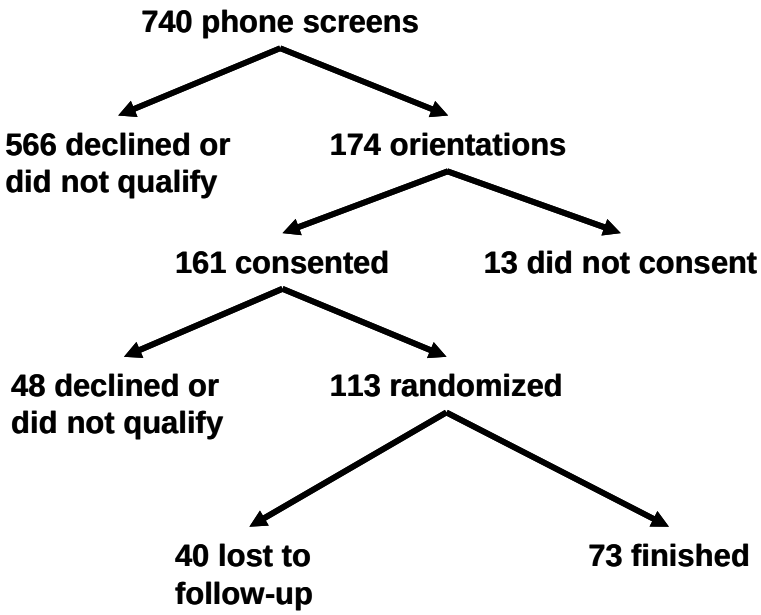

Table 1. Projected and actual enrollment by ethnicity and race

Race/Ethnic Category	Actual Enrollment	% Total	Projected Enrollment	% Total
RACE				
American Indian/ Alaskan Native	3	2	1	1
Asian	3	2	3	3
Native Hawaiian/Other Pacific Islander	0	0	0	0
Black/African American	2	2	6	6
White	99	89	92	90
Other/Hispanic	6	5	0	0
Total	**113**		**102**	
ETHNICITY				
Hispanic	15	13	20	20
Non-Hispanic	98	87	82	80
Total	**113**		**102**	

Table 2. Baseline characteristics of all subjects randomized to a treatment group, mean (sd)

	treatment group		
	ibuprofen/placebo	placebo/ibuprofen	placebo/placebo
n	38	39	36
n, on contraceptives (%)	24 (63)	23 (59)	22 (61)
age, y	30.1 (5.0)	29.6 (4.7)	31.6 (5.5)
weight, kg	65.9 (8.3)	64.3 (9.3)	65.6 (9.3)
height, cm	165.3 (7.2)	163.5 (6.1)	165.9 (8.3)
fat mass, kg	21.3 (5.3)	21.2 (7.0)	22.3 (6.6)
fat-free mass, kg	44.6 (4.6)	43.1 (4.4)	43.3 (5.0)
total BMC, g	2359 (324)	2217 (335)	2268 (347)
BMD, g/cm^2			
lumbar spine	1.128 (.141)	1.075 (.144)	1.106 (.131)
total hip	1.010 (.107)	0.968 (.114)	0.959 (.119)
femoral neck	0.910 (.133)	0.892 (.122)	0.870 (.113)
femoral trochanter	0.758 (.099)	0.733 (.114)	0.738 (.098)
femoral shaft	1.190 (.114)	1.130 (.133)	1.125 (.150)
VO_2max, mL/min/kg	33.5 (3.9)	33.4 (6.2)	31.5 (4.5)
HRmax, beats/min	190 (10)	189 (11)	189 (10)
RERmax	1.10 (.04)	1.10 (.06)	1.11 (.06)
energy intake, kcal/d*	1659 (328)	1648 (336)	1669 (399)
protein, g/d	75 (16)	67 (17)	67 (14)
fat, g/d	60 (20)	59 (22)	61 (19)
carbohydrate, g/d	197 (40)	210 (65)	207 (54)
calcium intake, mg/d	868 (351)	818 (320)	840 (289)

* dietary data were available for only 18 participants in each treatment group

Table 3. Baseline characteristics of subjects who completed the intervention, mean (sd)

	treatment group		
	ibuprofen/placebo	placebo/ibuprofen	placebo/placebo
n	23	21	29
n, on contraceptives (%)	13 (57)	11 (52)	19 (66)
age, y	29.9 (5.8)	30.1 (5.0)	32.2 (5.5)
weight, kg	65.8 (8.7)	63.6 (8.3)	66.3 (9.3)
height, cm	165.2 (8.2)	164.6 (5.9)	165.6 (9.1)
fat mass, kg	20.7 (5.3)	20.4 (6.2)	22.5 (6.2)
fat-free mass, kg	45.0 (4.6)	43.2 (4.7)	43.8 (5.2)
total BMC, g	2341 (312)	2229 (309)	2290 (364)
BMD, g/cm^2			
lumbar spine	1.106 (.131)	1.069 (.133)	1.092 (.138)
total hip	1.008 (.104)	0.980 (.104)	0.962 (.127)
femoral neck	0.905 (.126)	0.907 (.110)	0.867 (.118)
femoral trochanter	0.760 (.096)	0.748 (.111)	0.743 (.103)
femoral shaft	1.186 (.112)	1.138 (.116)	1.128 (.160)
VO_2max, mL/min/kg	34.2 (3.8)	33.9 (6.1)	31.4 (4.2)
HRmax, beats/min	191 (8)	188 (13)	189 (10)
RERmax	1.10 (.04)	1.12 (.05)	1.11 (.06)
energy intake, kcal/d*	1746 (348)	1753 (296)	1699 (344)
protein, g/d	79 (18)	68 (16)	68 (14)
fat, g/d	63 (20)	62 (18)	62 (18)
carbohydrate, g/d	208 (41)	231 (50)	210 (48)
calcium intake, mg/d	876 (395)	965 (300)	869 (241)

* dietary data were available for 12, 11, and 14 participants in the ibuprofen/placebo, placebo/ibuprofen, and placebo/placebo groups, respectively

Table 4. Baseline characteristics of subjects compliant to the intervention, mean (sd)

	treatment group		
	ibuprofen/placebo	placebo/ibuprofen	placebo/placebo
n	17	14	23
n, on contraceptives (%)	9 (53)	7 (50)	14 (61)
age, y	30.8 (6.2)	32.1 (5.0)	33.2 (5.3)
weight, kg	66.5 (9.3)	64.9 (9.4)	67.0 (9.8)
height, cm	165.0 (8.5)	165.2 (5.7)	167.2 (7.8)
fat mass, kg	21.5 (5.2)	21.0 (7.3)	22.5 (6.7)
fat-free mass, kg	45.0 (5.1)	43.8 (5.2)	44.5 (5.1)
total BMC, g	2307 (306)	2235 (350)	2377 (322)
BMD, g/cm^2			
lumbar spine	1.092 (.124)	1.061 (.152)	1.130 (.122)
total hip	1.004 (.112)	0.978 (.116)	0.982 (.128)
femoral neck	0.896 (.127)	0.915 (.125)	0.886 (.114)
femoral trochanter	0.755 (.105)	0.754 (.127)	0.760 (.098)
femoral shaft	1.184 (.117)	1.128 (.130)	1.150 (.166)
VO_2max, mL/min/kg	33.7 (3.8)	34.0 (7.1)	32.0 (4.4)
HRmax, beats/min	190 (9)	185 (11)	190 (10)
RERmax	1.10 (.04)	1.12 (.05)	1.12 (.06)
energy intake, kcal/d*	1715 (386)	1776 (263)	1803 (288)
protein, g/d	74 (19)	70 (13)	70 (13)
fat, g/d	63 (20)	62 (18)	64 (16)
carbohydrate, g/d	200 (45)	230 (46)	230 (40)
calcium intake, mg/d	905 (384)	1105 (186)	991 (202)

* dietary data were available for 9, 6, and 9 participants in the ibuprofen/placebo, placebo/ibuprofen, and placebo/placebo groups, respectively

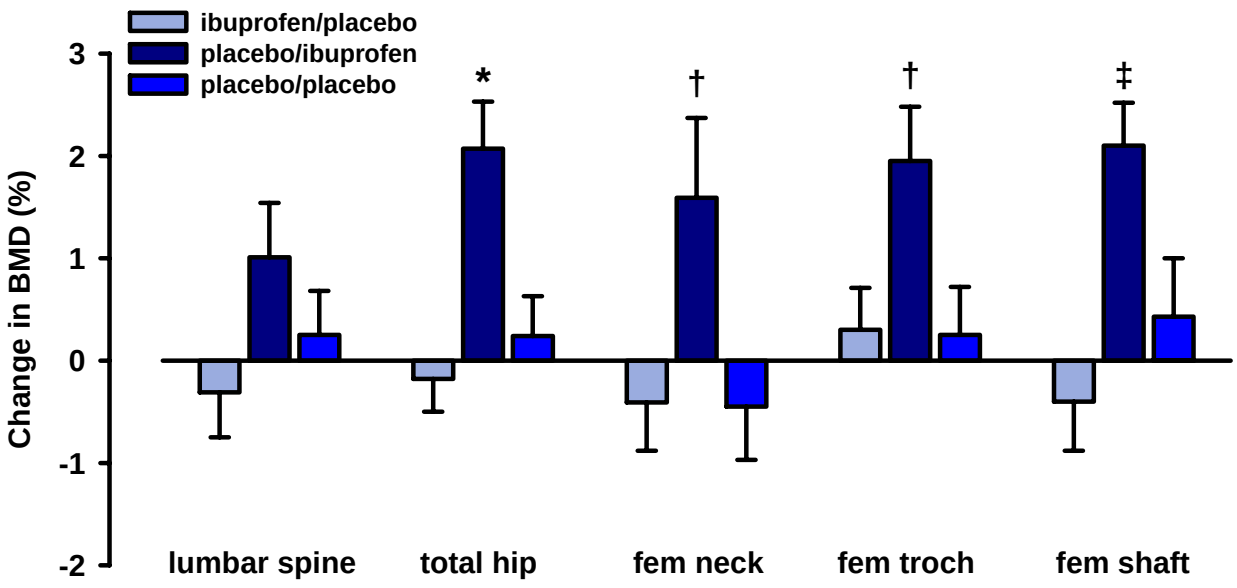

Figure 2. Changes in bone mineral density (BMD) in response to exercise training in women who were compliant to the intervention (* plac/ibup vs ibup/plac and plac/plac; † plac/ibup vs plac/plac; ‡ plac/ibup vs ibup/plac; all $p < 0.05$).

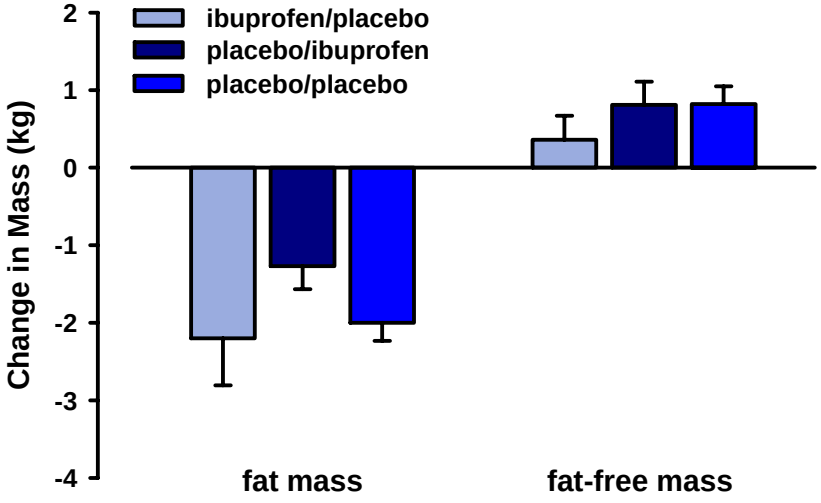

Figure 3. Changes in fat mass and fat-free mass in response to exercise training in women who were compliant to the intervention.

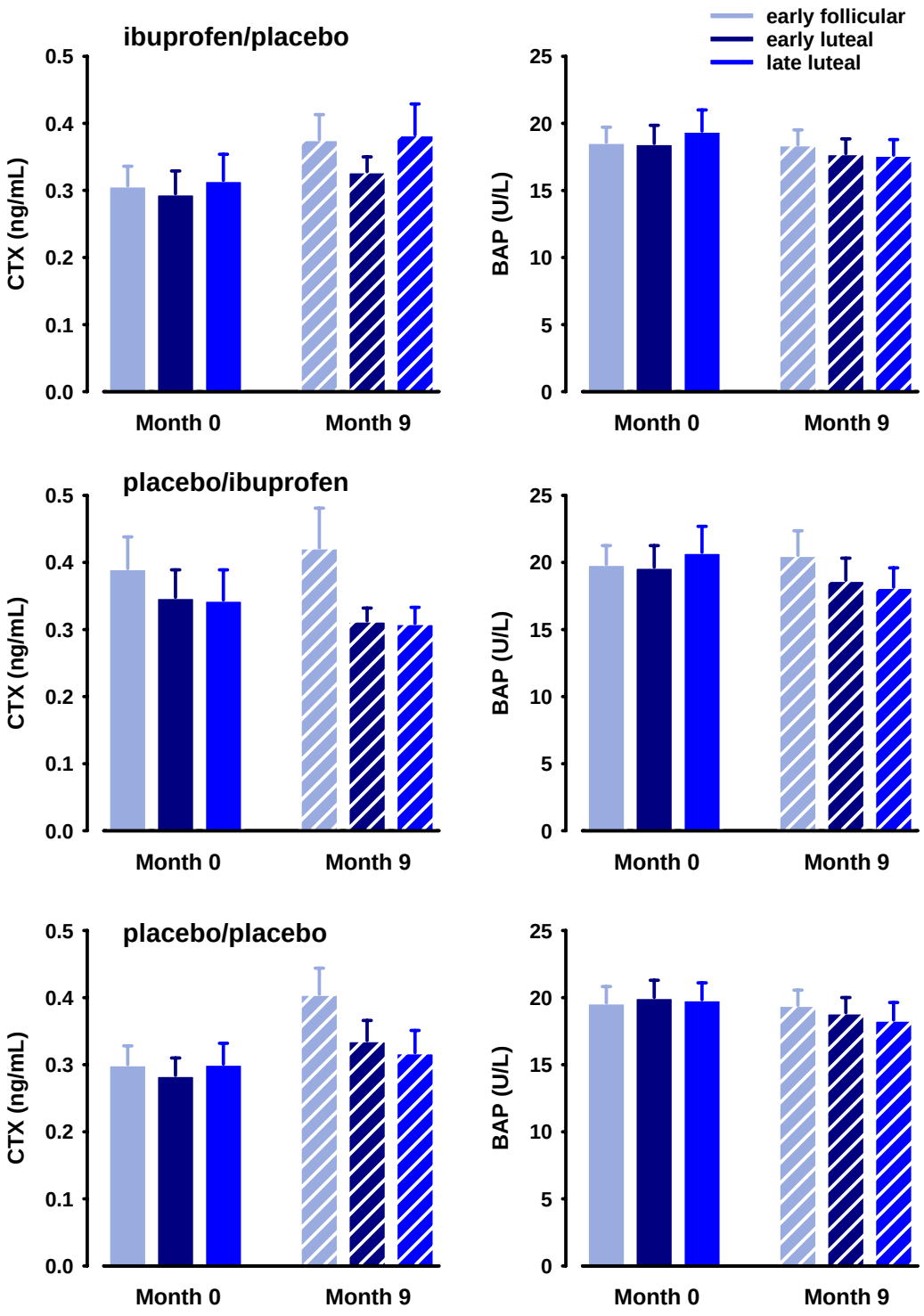

Figure 4. Markers of bone resorption (CTX) and formation (BAP) across the menstrual cycle before and after 9 months of exercise training.

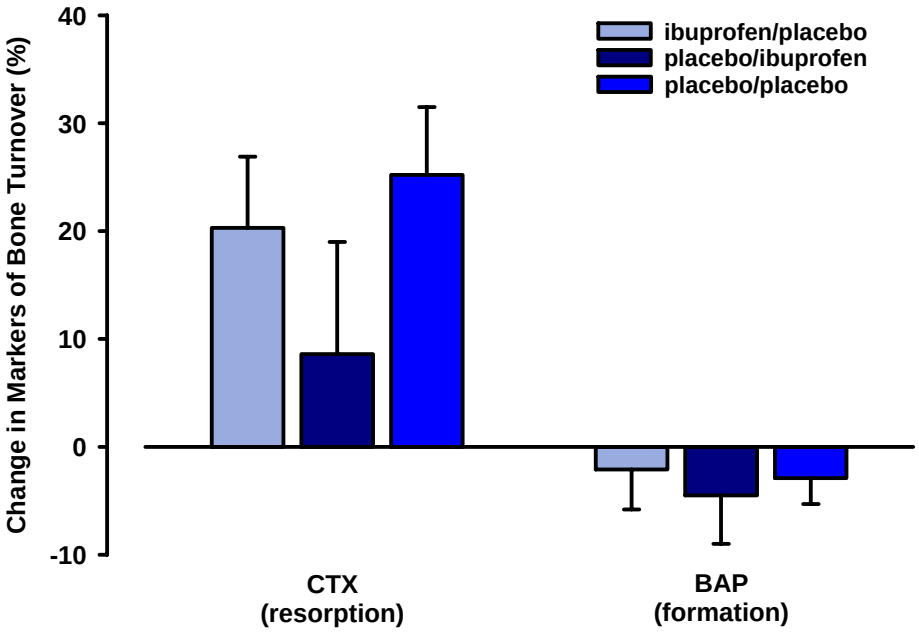

Figure 5. Changes in serum markers of bone resorption and formation in response to exercise training in women who were compliant to the intervention.

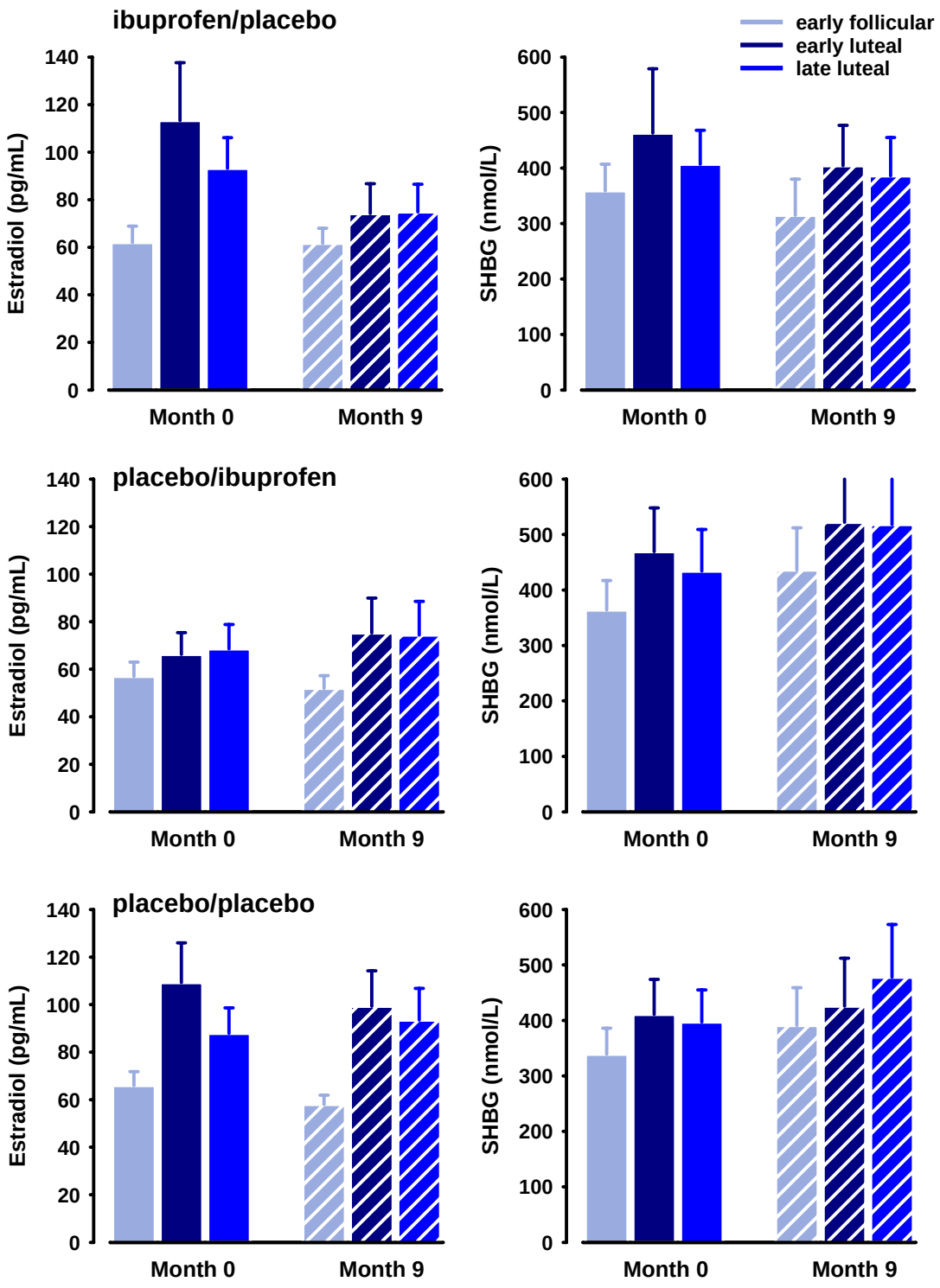

Figure 6. Serum estradiol and sex hormone binding globulin (SHBG) levels across the menstrual cycle before and after 9 months of exercise training.

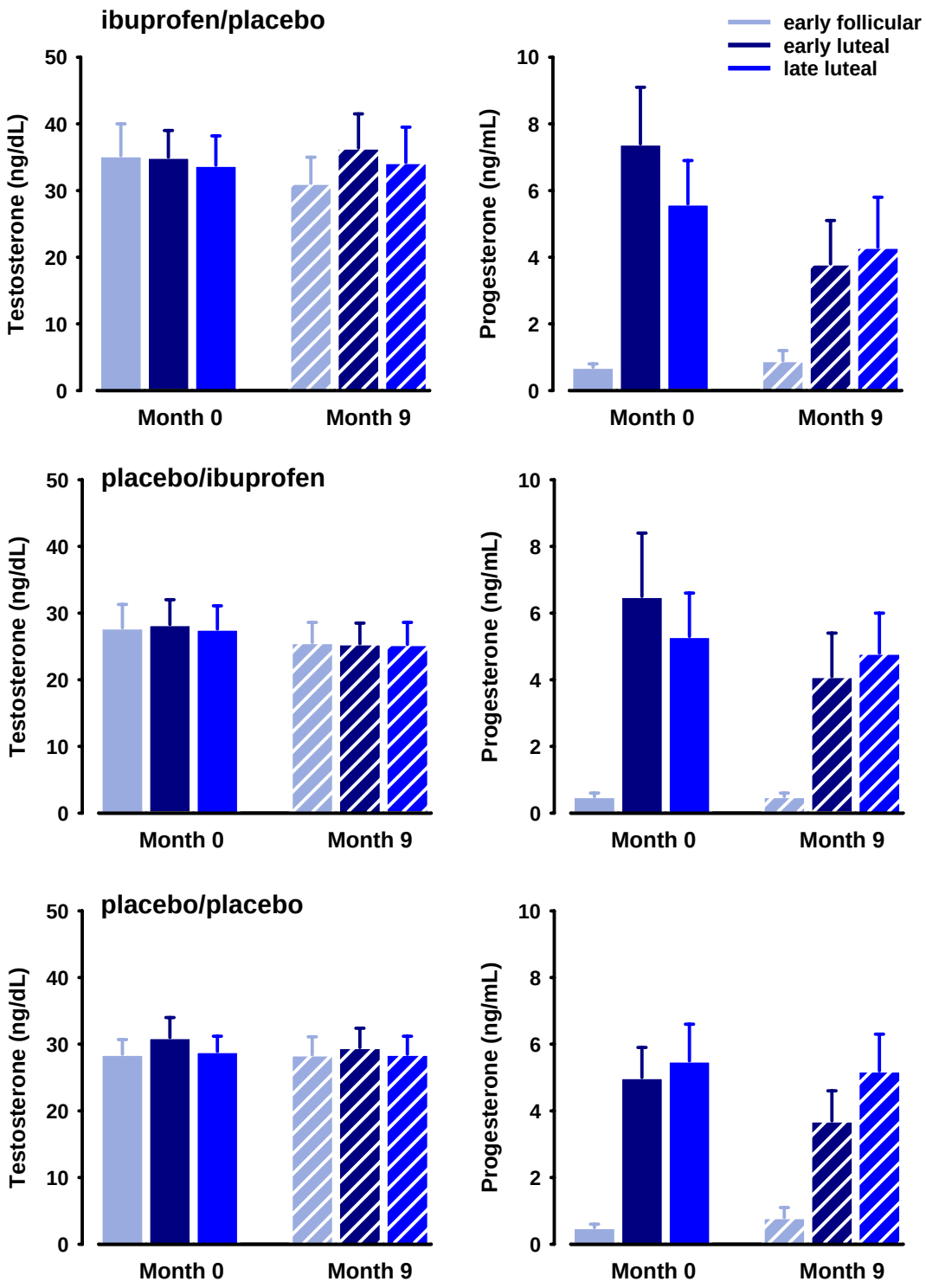

Figure 7. Serum testosterone and progesterone levels across the menstrual cycle before and after 9 months of exercise training.

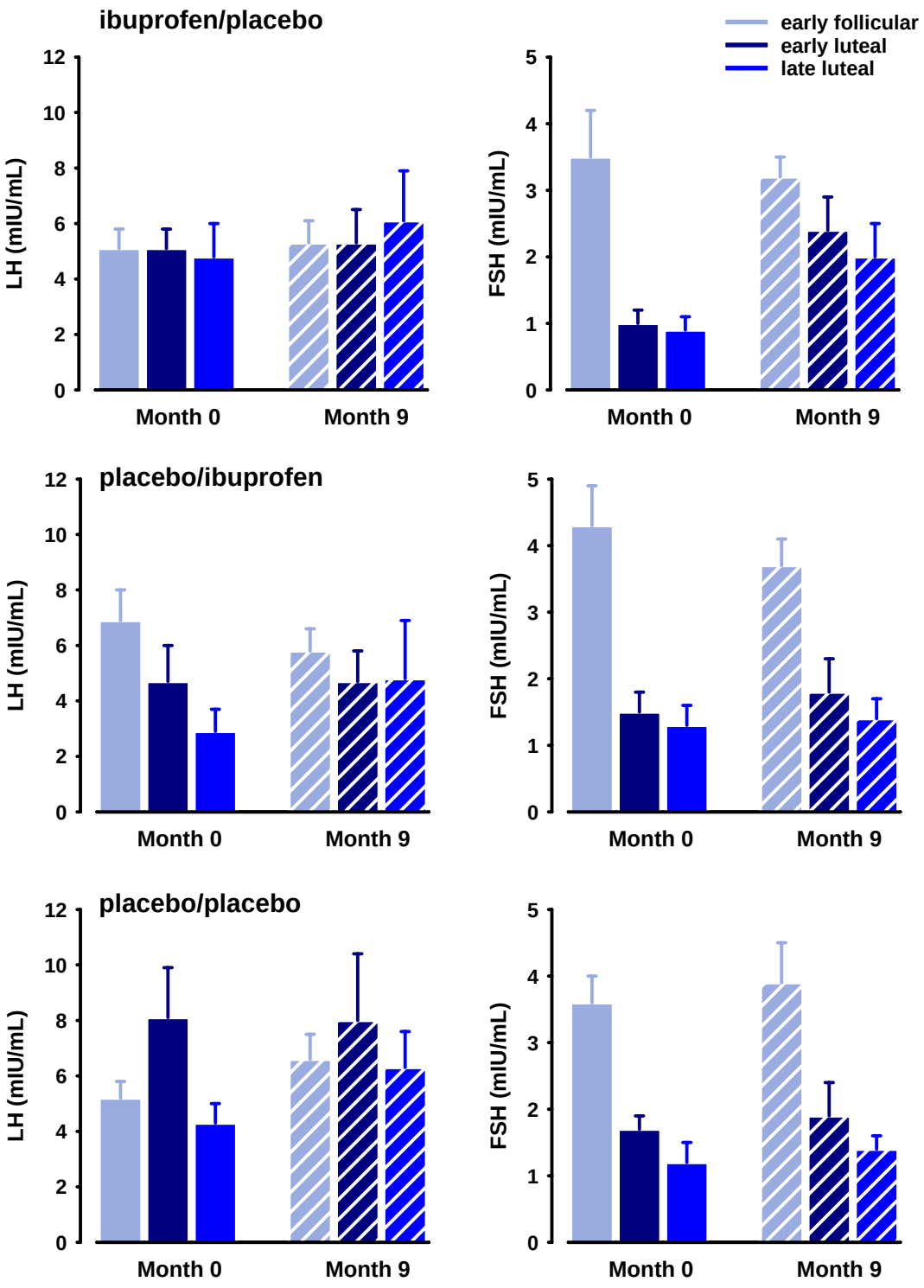

Figure 8. Serum luteinizing hormone and follicle stimulating hormone levels across the menstrual cycle before and after 9 months of exercise training.

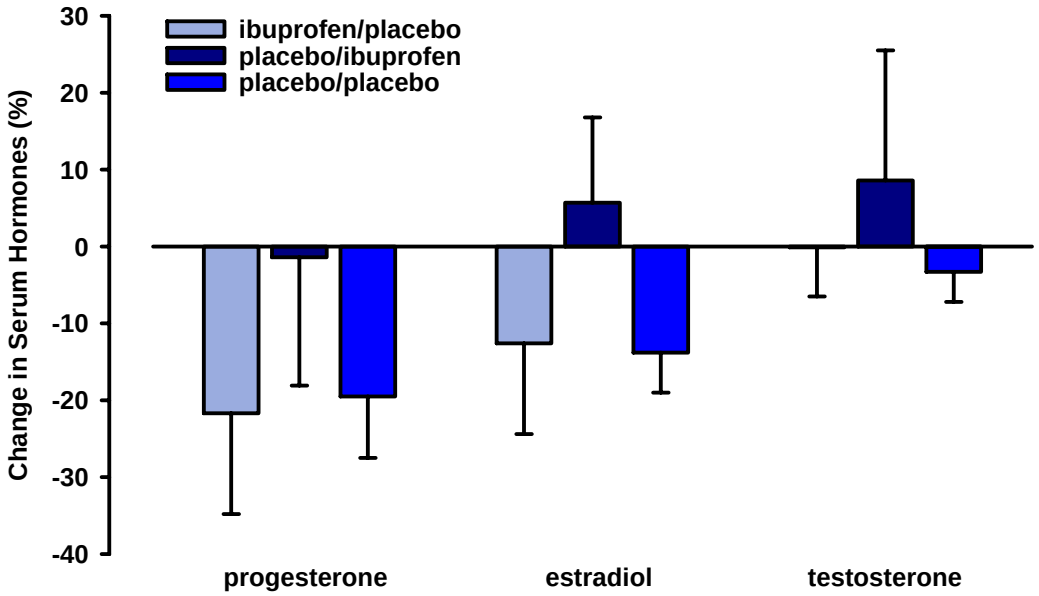

Figure 9. Relative changes in serum sex hormone levels in response to exercise training in women who were compliant to the intervention.

Table 5. Changes in cardiovascular fitness and energy intake in response to exercise training

	ibuprofen/placebo	placebo/ibuprofen	placebo/placebo
VO_2max, mL/min/kg	3.1 (3.1)	2.1 (5.2)	3.4 (2.0)
HRmax, beats/min	-4 (5)	-2 (8)	-3 (6)
RERmax	-0.04 (.05)	-0.05 (.07)	-.01 (.07)
Energy intake, kcal/d	-96 (422)	105 (358)	-63 (290)
protein, g/d	-5 (7)	6 (11)	4 (12)
fat, g/d	-6 (28)	1 (17)	2 (26)
carbohydrate, g/d	-11 (34)	-2 (52)	-20 (67)
Calcium intake, mg/d	-95 (355)	-31 (379)	248 (373)

VO_2max=maximal aerobic power; HRmax=maximal heart rate; RERmax=maximal respiratory exchange ratio; values are mean (sd)